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14. ABSTRACT Spinal cord injury is a devastating and debilitation condition that can induce depression and anxiety in the acute and chronic setting. There is general agreement in the medical community that these psychological health issues should be treated, mainly with administration of antidepressant medications. When considering which antidepressant drug to use, many physicians give venlafaxine, yet what remains unclear is the effect of this therapy on plasticity, functional rehabilitation and long-term psychological health after SCI. We are, therefore, using a rat model of SCI to study each of these variables. We report the exercise rehabilitation after SCI induced increases in hind limb locomotor recovery with no significant changes in allodynia or lower urinary tract function. Also, exercise rehabilitation induced an increase in immobility time as measured in the Porsolt forced swim, which was found to be related to swimming ability. Thus, we have found that exercise rehabilitation increases functional recovery and on-going studies will evaluate the effect of venlafaxine on this improvement.					
15. SUBJECT TERMS Spinal cord injury, depression, exercise, recovery					
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INTRODUCTION:

Spinal cord injury (SCI) is a devastating and debilitating condition that affects an estimated 227,080 to 300,938 persons in the United States with approximately 12,000 new cases occurring each year. Many studies suggest that symptoms of depression and anxiety are common in both the acute and chronic setting after SCI with an estimated 20-43% of persons with SCI being at risk of developing a depressive disorder during rehabilitation and 16-60% of persons with SCI experiencing depression of clinical significance when living in the community. There is general agreement in the clinical community that these psychological complications are associated with poor outcome; thus, clinicians compassionately strive to treat depression in persons with SCI typically by prescribing antidepressants. When considering which antidepressant agent to use, the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine is often chosen because of its low side effect profile, its low potential for drug interactions, and its potential analgesic properties. What remains unclear is the effect of SNRI therapy on plasticity, functional rehabilitation, and long-term psychological health after SCI. Importantly, each of these variables can be systematically evaluated in pre-clinical SCI models to help fill in this critical gap in knowledge, as we are investigating here.

BODY:

Upon completion of 1 year of this study, we have made good research progress. Our progress in this first year is detailed according to the tasks in the Statement of Work. The tasks are listed below and italicized for emphasis. Our progress to date for each task then follows.

Task 1. Enable SCI and Depression Research Project. (Months 1-2)

1.a. Order critical supplies and equipment

1.b. Prepare and submit IACUC documents for protocol approval.

Milestone 1: Animal Use approval.

1.c. Order rats on approved IACUC protocol.

1.d. Train staff for the performance of the spinal cord injury.

1.e. Train staff for the performance of bladder volume measures.

1.f. Train staff for the performance of the panel of in vivo and ex vivo tests to be employed for the study.

The UAB IACUC approved this animal use protocol in June 2010 and renewed the approval in June 2011. The approval notice is attached in the appendix. Thus, milestone 1 is completed. Additionally, the laboratory staff working with this project have been trained in the post-operative care of the spinal cord injured animals as well as in the assessment of bladder volumes and other evaluation measures. Thus, task 1 was completed on schedule.

Task 2. Establish swim exercise rehabilitation protocol. (Months 3-6)

- 2.a. Acclimate rats to swim exercise rehabilitation.
- 2.b. Pre-test rat hindlimb function using CatWalk gait analysis and Louisville swim test.
- 2.c. Pre-test rat sensory function using tail flick and von Frey tests.
- 2.d. Perform spinal cord injury surgery.
- 2.e. Administer post-operative medications twice daily for 7 days.
- 2.f. Measure body weight and urine volumes.
- 2.g. Test rat hindlimb function using Basso, Beattie, Bresnahan open-field test and CatWalk analysis weekly for the duration of the study.
- 2.h. Test rat sensory function using tail flick and von Frey tests weekly for the duration of the study.
- 2.i. Initiate swim exercise rehabilitation four weeks post-SCI and continue for four weeks.
- 2.j. Test rat hindlimb function using the Louisville swim test weekly beginning the first week of rehabilitation.

Milestone 2. Established a swim exercise rehabilitation protocol.

We have built the swim exercise tank and established and evaluated a swim exercise rehabilitation protocol. For the experiment to established and validate the swim exercise protocol, ten adult male rats received a moderately severe mid-thoracic (T10) SCI induced with the Infinite Horizons SCI impactor. Rats were then randomly divided into 2 cohorts: rats that received exercise therapy (n=5) and rats that received no exercise therapy (n=5), beginning 7 days post-SCI. Exercise therapy was conducted as swimming rehabilitation which included swim training (4 days/week for 4 weeks) and was administered in a Plexiglas pool (60" long, 7"

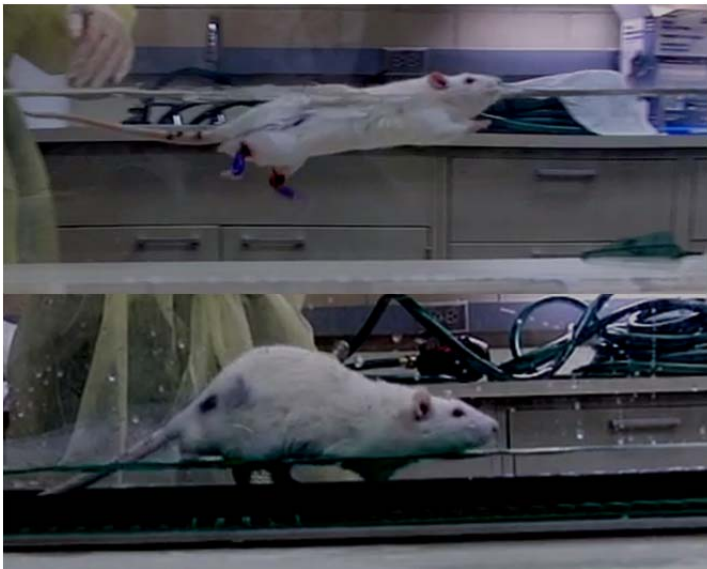


Figure 1: Upper panel: Image of behavioral training and assessment using the Louisville Swim task. Lower panel: Image of body weight supported walking (Shallow Water Walking).

wide, and 12" deep) to those animals in the rehabilitation group. During the last two weeks of rehabilitation, shallow water walking was added for body weight supported training, and rats walked with 40% weight support (figure 1). All rehabilitation and evaluation sessions were recorded with a digital camera for subsequent evaluation and analysis. To better visualize each paw and the trunk orientation of the animal, magic marker was applied to the feet and fur.

Animals were inspected a minimum of twice daily and days to voluntary micturition was evaluated. Weekly after SCI and rehabilitation, we assessed hind limb function using the

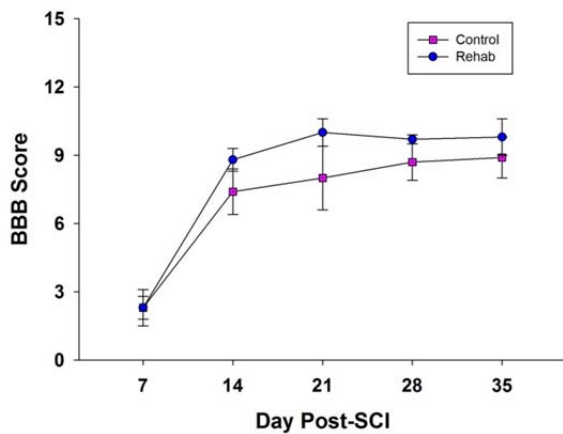


Figure 2: Quantification of BBB Open Field Locomotor Scale. A trend is shown with increased swim scores for the rehabilitation animals, but there is no significant difference between the rehabilitation and control groups. N=5/group.

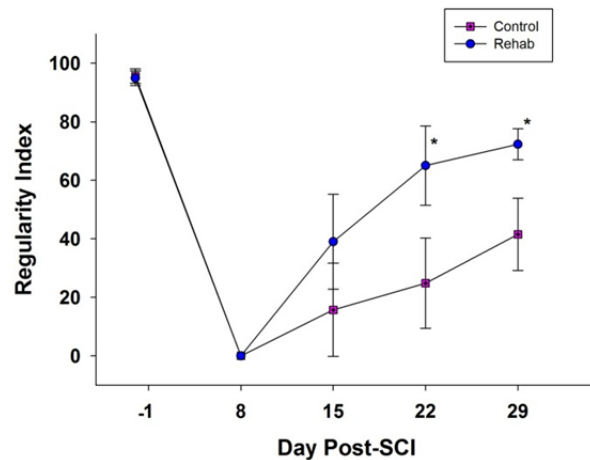


Figure 3: Quantification of CatWalk® gait task. Rehabilitation animals had improved locomotor function shown by increased interlimb coordination. N=5/ group. *=p<.05.

Basso, Beattie, Bresnahan (BBB) Open Field Locomotor Scale¹. As previously described^{2,3}, rats were placed in an open field and hind limb movements and locomotion were scored by two raters skilled in the evaluation of the categories. We found that the exercise rehabilitation beginning at 7 days post-SCI induced an increase BBB scores as compared to the control group, but this did not reach statistical significance (figure 2). Rats were also assessed using the Catwalk® gait analysis system⁴. For this analysis each rat crossed a glass walkway and paw

prints were recorded in real time and digitized for subsequent analysis. Paw prints were analyzed using kinematic criteria as previously described⁵. We found that post-SCI exercise rehabilitation induced a significant increase in fore-hind limb coordination as indicated by improved regularity index scores (figure 3). Additionally, the Louisville Swim Scale was used to evaluate hind-limb function⁶. For this test, each rat swam laps in a Plexiglass pool for 4 minutes and was recorded with a digital camera. Recordings were analyzed for hindlimb movement, forelimb dependency, and body angle in the water as previously described⁶. We found that post-SCI exercise rehabilitation training induced an increase in the hindlimb motor performance as compared to controls that

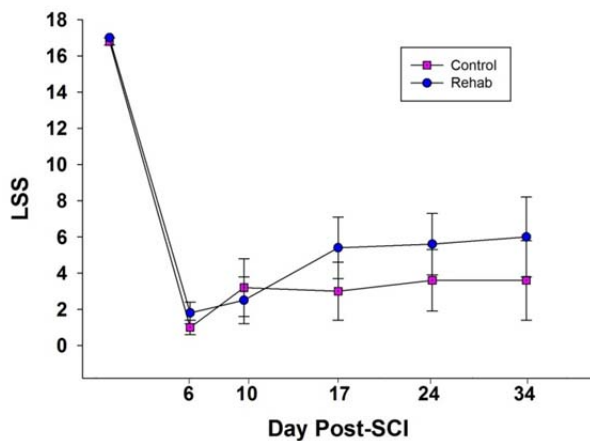


Figure 4: Quantification of Louisville Swim Scale. A trend is shown with increased swim scores for the rehabilitation animals, but there is no significant difference between the rehabilitation and control groups. N=5/ group.

received no rehabilitation training, but this trend did not reach statistical significance (figure 4). Hypersensitivity to a non-painful stimulus, allodynia, was evaluated weekly using sensitivity to Von Frey filaments. We found no differences in the hindlimb sensitivity as indicated by the paw withdraw threshold (PWT) between treatment groups (figure 5). Similarly, we found no difference in days to voluntary micturition between treatment groups (figure 6). Lastly, depression/anxiety was evaluated using the Porsolt forced swim test^{7, 8} on post-SCI day 36. For this evaluation, rats were placed in a cylinder with water and monitored for immobility time, which is associated with depression^{7, 8}. Unexpectedly, we found that rats in the rehabilitation exercise group had a non-statistically significant increase in immobility time as compared to animals in the no-rehab control group (figure 7). We observed some rats swim better than others and that this may have affected the Porsolt test. Thus, we conducted a Pearson correlation matrix between percentage of immobility time and LSS (figure 8). We found a significant positive correlation between the two measures ($r=0.645$) which indicated that rats that swim better have greater immobility time.

In summary, we found that post-SCI exercise rehabilitation stimulated: 1) Increased hind limb locomotor recovery as measured by the CatWalk® Gait Analysis, 2) a trend of increased locomotor recovery in the Louisville Swim Scale and BBB Open Field Test; 3) increased immobility time, as measured by the Porsolt Forced Swim Test for depression 4) no significant changes in allodynia or bladder control. We plan to add more animals to this study to increase statistical power of this analysis. Taken together, these preliminary results demonstrate that we have achieved task 2 and all the associated subtasks and that we now have established and confirmed a swim exercise rehabilitation protocol.

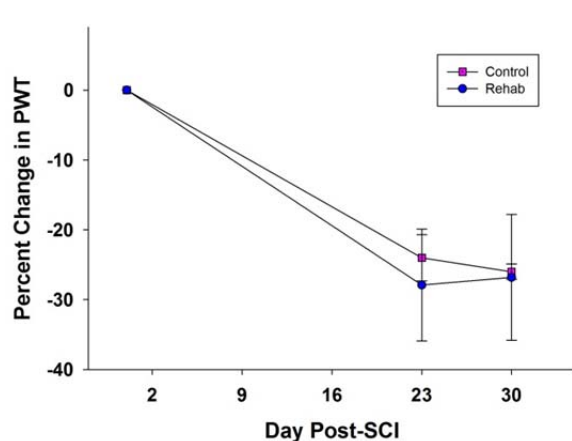


Figure 5: Rehabilitation exercise showed no significant effect on allodynia as measured using Von Frey filaments to determine the paw withdraw threshold (PWT). N= 5/group.

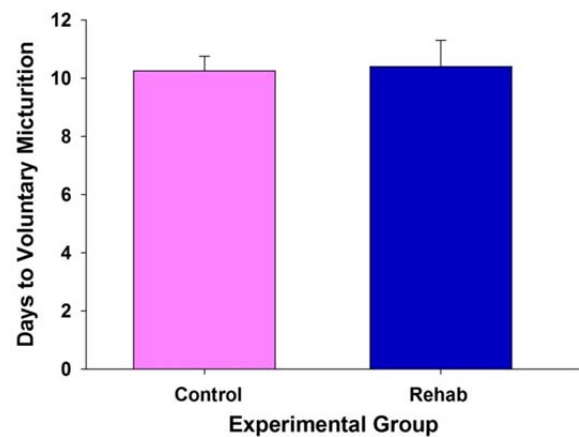


Figure 6: Rehabilitation exercise showed no significant effect on lower urinary function as measured by days to voluntary micturition. N= 5/group.

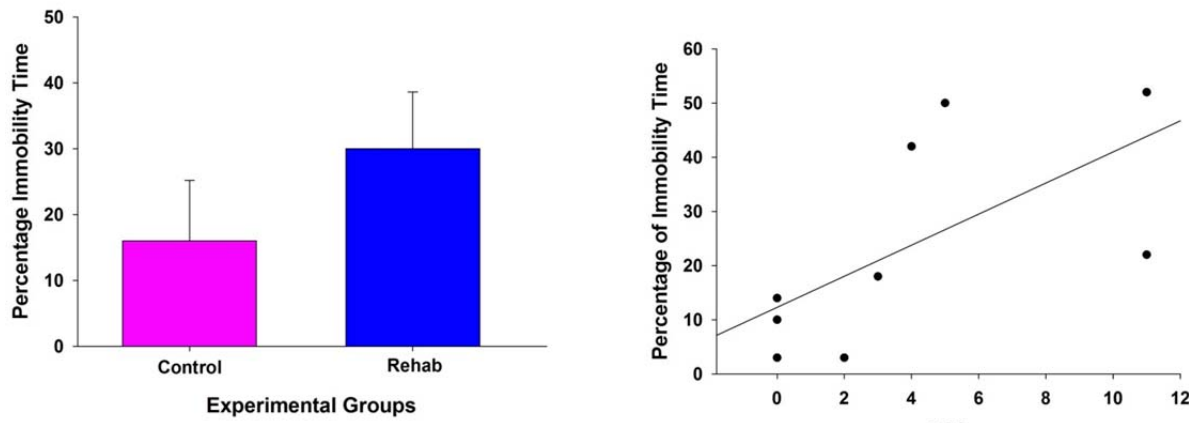


Figure 7. Effects of Exercise Rehabilitation on the Porsolt Forced Swim Test for Depression. *Left Panel:* Exercise Rehabilitation is associated with increased immobility time in the Porsolt Forced Swim Test, yet this did not reach statistical significance. *Right Panel:* However, increased swimming ability, as indicated by the Louisville Swim Scale (LSS), is correlated with greater immobility time in the water ($r = 0.645$), as indicated by a Person correlation matrix.

Task 3. Initiate venlafaxine treatment in rat spinal cord injury model. (Months 6-18, ongoing and concurrent with other tasks)

- 3.a. Acclimate rats to gavage procedure for two weeks using sugar water.
- 3.b. Acclimate rats to swim exercise therapy.
- 3.c. Pre-test rat psychological health using Porsolt forced swim test for depression and elevated plus maze for anxiety.
- 3.d. Perform spinal cord injury surgery.
- 3.e. Administer post-operative medications twice daily for 7 days.
- 3.f. Measure body weight and urine volume once daily.
- 3.g. Test psychological health prior to the initiation of venlafaxine treatment (DPO 12 & 13) using Porsolt forced swim test for depression and elevated plus maze for anxiety.
- 3.h. Initiate venlafaxine treatment 3 times daily via gavage (25 mg/dose) starting four weeks post-SCI and continuing for four weeks.
- 3.i. Initiate swim exercise rehabilitation starting four weeks post-SCI and continuing for four weeks using the protocol established in Task 1.
- 3.j. Test psychological health at the end of the treatment phase using the Porsolt forced swim test for depression and the elevated plus maze for anxiety.

Milestone 3. Determined the effect of venlafaxine ± swim exercise rehabilitation treatment on depression and anxiety in a rodent model of SCI.

Task 4. Assess the effect of venlafaxine ± swim exercise rehabilitation on hindlimb functional recovery in a rat spinal cord injury model. (Months 6-18, ongoing and concurrent with other tasks)

- 4.a. Acclimate rats to swim exercise rehabilitation.

- 4.b. Pre-test rat hindlimb function using CatWalk gait analysis and Louisville swim test.
- 4.c. Perform spinal cord injury surgery.
- 4.d. Administer post-operative medications twice daily for 7 days.
- 4.e. Measure body weight and urine volumes daily.
- 4.f. Test rat hindlimb function using Basso, Beattie, Bresnahan open-field test and CatWalk analysis weekly for the duration of the study.
- 4.g. Initiate venlafaxine treatment 3 times daily via gavage (25 mg/dose) starting 4 weeks post-SCI and continuing for 4 weeks.
- 4.h. Initiate swim exercise rehabilitation four weeks post-SCI and continue for four weeks.
- 4.i. Test rat hindlimb function using the Louisville swim test weekly beginning the first week of rehabilitation.
- 4.j. Perfuse rats at the end of the study and collect spinal cord and brain tissue for histological and biochemical analysis.

Milestone 4. Determined the effect of venlafaxine \pm swim exercise rehabilitation treatment on hindlimb functional recovery in a rodent SCI model.

Task 5. Assess the effect of venlafaxine \pm swim exercise rehabilitation on sensory function in a rat spinal cord injury model. (Months 6-18, ongoing and concurrent with other tasks)

- 5.a. Acclimate rats to swim exercise rehabilitation.
- 5.b. Pre-test rat sensory function using tail flick and von Frey tests.
- 5.c. Perform spinal cord injury surgery.
- 5.d. Administer post-operative medications twice daily for 7 days.
- 5.e. Measure body weight and urine volumes daily.
- 5.f. Test rat sensory function using tail flick and von Frey tests weekly for the duration of the study.
- 5.g. Initiate venlafaxine treatment 3 times daily via gavage (25 mg/dose) starting 4 weeks post-SCI and continuing for 4 weeks.
- 5.h. Initiate swim exercise rehabilitation four weeks post-SCI and continue for four weeks.
- 5.i. Perfuse rats at the end of the study and collect spinal cord and brain tissue for histological and biochemical analysis.

Milestone 5. Determined the effect of venlafaxine \pm swim exercise rehabilitation treatment on sensory function in a rodent SCI model.

Tasks 3, 4, and 5 are currently on-going. In an effort to limit scientific bias, we randomize the rats to the treatment groups and ensure that the investigators are naïve to the drug administration group of the animals. Therefore, we do not un-code the treatment groups in the middle of a study as would be necessary to produce graphs of the preliminary results. Notably, as there are only a few investigators working with this project, it is impossible to blind all investigators to the exercise group membership of the rats. Thus far, we have completed behavioral assessments for 15 animals. These behavioral assessments include the hindlimb function analysis (locomotion) with the BBB test, the LSS test, and the Catwalk. Additionally, we have completed the sensory function test for these 15 animals. Lastly, the Porsolt test has been conducted for this cohort. All of the animals in the first cohort were humanely euthanized and the spinal cord tissue was perfused and extracted. We then cryoprotected and embedded the tissue into OCT to make cryomolds so that this tissue can be used for task 6. We currently have another cohort of 15 rats

under investigation. Thus, we are in the middle of tasks 3, 4, and 5 and do not want to break the group membership of the animals for the data we have collected this far as to risk unnecessary bias being introduced into the study.

Task 6. Perform histological and biochemical analysis of spinal cord tissue. (Months 10-22 months, ongoing and concurrent with other tasks)

6.a. Slice paraformaldehyde perfused spinal cord tissue.

6.b. Quantify lesion size.

6.c. Stain spinal cord tissue slices with Cresyl violet for neuronal survival.

6.c. Quantitate surviving neurons.

6.d. Stain spinal cord tissue slices with myelin basic protein (MBP) for white matter sparing.

6.e. Quantify spared white matter.

6.f. Quantify axonal sprouting in spinal cord tissue.

6.f. Quantitate spinal cord tissue levels of serotonin and norepinephrine using ELISA assays.

6.g. Quantify levels of 5-HT and NE receptors at epicenter of lesion and in adjacent segments.

Milestone 6. Determined the effect of venlafaxine \pm swim exercise rehabilitation treatment on spinal cord plasticity

This task has not yet been started, but tissue is being collected in preparation for this task.

Task 7. Prepare peer-reviewed manuscript(s) and platform/poster presentation at national meeting to report results. (Month 22-24)

Milestone 7. Completed study and reported results to peer community.

This task has not yet been started.

KEY RESEARCH ACCOMPLISHMENTS:

We found that post-SCI exercise rehabilitation stimulated:

- Increased hind limb locomotor recovery as measured by the CatWalk ® Gait Analysis,
- A trend of increased locomotor recovery in the Louisville Swim Scale
- A trend of increased locomotor recovery in the BBB Open Field Test
- Increased immobility time, as measured by the Porsolt Forced Swim Test for depression
- No significant changes in allodynia or bladder control

REPORTABLE OUTCOMES:

1. Results obtained from task two are to be presented at the Association of Academic Physiatrists Annual Meeting in February 2012. The abstract for this presentation is attached as appendix 2.

CONCLUSION:

We have made good progress and are moving according to plan in our investigation of the effects of post-SCI administration of antidepressants and exercise rehabilitation on functional recovery and psychological health after SCI in a rat model. We do not anticipate any obstacles to the continuation of this research project.

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APPEDICES:

Appendix 1: IACUC Approval Form

Appendix 2: Abstract for AAP meeting.



NOTICE OF RENEWAL

DATE: May 11, 2011

TO: CANDACE L. FLOYD, Ph.D.
SRC -547 7330
FAX: (205) 934-5086

FROM: 
Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: Title: Effect of Antidepressant Therapy on Psychological Health, Rehabilitation, Plasticity, and Functional Recovery in a Rodent Model
Sponsor: Department of Defense
Animal Project Number: 110609180

As of June 23, 2011, the animal use proposed in the above referenced application is renewed. The University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) approves the use of the following species and numbers of animals:

Species	Use Category	Number in Category
Rats	B	110

Animal use must be renewed by June 22, 2012. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 110609180 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at (205) 934-7692.

FUNCTIONAL AND PSYCHOLOGICAL RECOVERY FOLLOWING SPINAL CORD INJURY: SYSTEMATICALLY EVALUATING THE EFFECTS OF EXERCISE REHABILITATION IN A RAT MODEL

AUTHORS: Anna M. Edmiston, Sarah M. Kezar, Don E. McCormick, Candace L. Floyd, Ph.D.

AFFILIATIONS: Department of Physical Medicine and Rehabilitation. University of Alabama at Birmingham; supported by AAP RREMS and W81XWH-10-1-0839

ABSTRACT:

Objective: In this study, we used a clinically-relevant rat model of spinal cord injury (SCI) to systematically evaluate the effect of post-SCI exercise rehabilitation on locomotor function, bladder function, neuropathic pain, and depression.

Design: Ten adult male rats received an equivalent contusion SCI and then were randomly assigned to receive either swimming rehabilitation (swim training 4 weeks, with additional shallow water walking weeks 3-4) or no rehabilitation. Hind limb locomotion was evaluated weekly post-SCI using the Basso, Beattie, Bresnahan (BBB) scale, the Louisville Swim Scale (LSS), and catwalk kinematic analysis. We assessed bladder function by days to micturition. Von Frey filaments were used to measure allodynia. Depression was measured using the Porsolt forced swim test, where increased immobility time is associated with increased depression.

Results: Improved hind limb locomotion in the rehabilitation group versus the control group was measured according to the BBB scale, the LSS scale, and catwalk kinematic analysis. No significant differences in allodynia or bladder control were observed between groups. We found that rats in the rehabilitation group had increased immobility time versus the control group.

Conclusions: SCI typically results in functional impairment, and approximately 30% of persons with SCI suffer from depression. Depression is often treated with serotonergic antidepressants, but recent trials have shown exercise to be more effective than antidepressants in alleviating depression. Rats in the rehabilitation group had increased immobility time versus the control group, which indicates greater depression in able-bodied rats. However, animals with greater swimming ability exhibited greater immobility time in the forced swim test, which may obfuscate the interpretation in paraplegic animals. Exercise rehabilitation improved functional recovery in all three testing parameters. Future studies will need to evaluate additional measures of psychological health and evaluate the cellular substrates involved in the exercise-induced effects on functional recovery and psychological health.